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STUDIES OF AN ICHTHYOSPORIDIAN-LIKE PARASITE OF OCEANPOUT

(*ZOARCES ANGUILLARIS*)

By

Leslie A. Sandholzer, Thomas Nostrand, and Leo Young

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Explanatory Note

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CONTENTS

	Page
Introduction	1
Literature	2
Scope of Investigation	2
Pathology of the Parasitized Fish	3
Detection of Parasite	3
Incidence of Infection	3
Spread of Lesions in Excised Tissue	3
Methods of Eliminating Parasites	3
Toxicity Studies	3
Experimental Methods	4
Pathology	4
Detection of Parasitic Lesions	4
Incidence of Infection	4
Spread of Lesions	5
Toxicity Tests	5
Results	5
Pathology of Parasitized Fish	5
Detection of Lesions in Fillets	6
Incidence of Parasitism	6
Spread of Lesions in Fillets	7
Toxicity Studies	8
Discussion	9
Summary	9
Literature Citations	12

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INTRODUCTION

One of the major developments resulting from the requirements of World War II has been the exploitation of previously unused sources of protein food. A most astonishing aspect of this program has been the rise in the use of oceanpout (Zoarces anguillaris), also known locally as eelpout, conger eel, congo eel, wolffish, ling, lingcod and muttonfish. Although this fish has been sold on the New York market in small quantities for at least 50 years, its commercial importance was negligible until 1943 (Market News Service Reports, 1942-43).

No receipts of oceanpout were reported from any of the markets in 1942. From February through April 1943, 217,855 pounds were received at Gloucester and Boston, Mass., and Portland, Maine. From May to October, landings were negligible. In November and December, an additional 7,235 pounds were landed at the same ports. At New Bedford, Mass., 3,279,129 pounds were brought in during 1943. In addition, numerous small landings were made at various other ports from Cape May, N. J., to Portland, Maine, and for the most part, such landings have not been included in the official statistics.

Total receipts of this fish on the New York City market for 1943 were 830,333 pounds. During the first three months of 1944 the receipts in New York City were 1,572,089 pounds. The value, calculated at three cents per pound, was $57,163 (see Amendment 27 to MPR 418 - Fishery Market News Report No. 68, 1944). Table 1 summarizes market statistics regarding oceanpout landings in 1943.

Although this rise of the oceanpout was sudden and spectacular to commercial importance in the United States, this should not be construed to indicate that it had not received consideration elsewhere as a food source. The European species have been found regularly for years in continental markets. A similar species long has been used as food by the Chinese. Furthermore, the possibility of increasing the use of the oceanpout as food had received the serious consideration of the Canadians during and immediately following World War I (Clemens, 1920).

To date fishing operations for the oceanpout have been restricted to the inshore waters of northeastern United States and southeastern Canada. In 1918, catches were made from Block Island to Nova Scotia (Clemens, 1920). The present fishing grounds extend from Ocean City, N. J. to Portland, Maine. From January through March the fish are caught 30 to 50 miles off shore, but as the weather moderates the best fishing is found further inshore. The season lasts from October to May of the following year.

Despite the demand for this fish as food, it became apparent early in 1943 that because of a protozoan infection in its flesh, the oceanpout fishery might undergo a decline which would be as meteoric as its rise. The parasite, Ichthyosporidium sp. (?) (Nigrelli, 1943; Fischthal, 1944) produces a lesion which is not only objectionable aesthetically, but if it were proved to be toxic, it would become a definite public health menace. Accordingly, the Technological Laboratory at College Park, Md., in cooperation with the Office of the Coordinator of Fisheries, undertook studies to determine the incidence of infection, the

spread of the parasite to fillets of non-infected fish, toxicity of parasitized fish, and related problems. This paper reports the data found.

LITERATURE

The genus Ichthyosporidium was first designated by Caullery and Mesnil (1905 b). They described two species found in marine fishes, Ichthyosporidium gastrophilum and Ichthyosporidium phymogenes. The former was restricted to the digestive tract of Motella mustella and Liparis vulgaris; the latter occurred in the subcutaneous tissue between the skin and muscle of Crenilabrus melops. Four years later, Robertson (1909) reported a species of Ichthyosporidium which caused a fatal disease in seatrout. The same author also described a similar infection of haddock. Because her findings closely resembled those seen in parasitized Zoarces, copies of the drawings have been included in this report (figs. 1 and 2).

Although Caullery and Mesnil described the parasites as found in the fish and the accompanying pathology, it was not until 1914 that the life cycle of one species Ichthyosporidium giganteum, was worked out by Swarczewsky (fig. 3, Kudo, 1939). This species is infectious for Crenilabrus melops. The same investigator also described Ichthyosporidium hertwigi which parasitizes the gills of Crenilabrus paro.

Wenyon (1926) makes the following statement regarding the genus: "The genus, Ichthyosporidium, Caullery et mesnil (1905), includes several species which infect fish. As in the case of the Microsporidiida, white nodules are produced in the tissues and on the gills and these are seen to consist of an encapsulated multinucleate plasmodium in which occur ovoid spores."

Kudo (1939) defines the genus as follows: "Genus Ichthyosporidium Caullery et Mesnil. In fish; often looked upon as Microsporidia, as the organism develops into large bodies in body muscles, connective tissue or gills, which appear as conspicuous "cysts" which are surrounded by a thick wall and contain numerous spores."

It would seem from the above literature that this genus is closely related to other protozoan genera, especially to the Microsporidia and Haplosporidia. However there seems to be some confusion as to the exact nature of the parasite, some workers considering it to be a fungus (Plehn, 1924). Fish (1934) has carefully reviewed the literature regarding this controversy but does not commit himself to either the protozoan or fungus classification, although he apparently favors the latter. Failure to observe hyphae in the lesions in Zoarces lead to the acceptance of the protozoan hypothesis.

SCOPE OF INVESTIGATION

The problem of this particular parasitism of Zoarces was first observed by Herrington and others in 1943, but there was no simple answer immediately available. Little was known of either the fish or the parasite, and there was an urgent demand for a rapid solution in the interest of the fishery. In order to meet this exigency a program was formulated which included the investigations listed below:

Pathology of the Parasitized Fish

This was investigated in order to obtain information as to how the parasite injured the host and to procure possible clues as to the manner of its spread in the host tissues.

Detection of Parasite

A rapid simple method had to be devised for determining the presence of lesions in oceanpout. Fortunately, the fish was being marketed as fillets. Methods of candling already had been developed for detecting Sphyrion lumpi in rosefish fillets (Firth, Nigrelli, Herrington, and Bearse, 1939) and it merely remained to modify the technic for application to field studies.

Incidence of Infection

It was necessary to obtain information as to the extent of the parasitism in the various areas where commercial catches were being made. This would not only indicate areas of heavy and light infestation but would also yield data regarding the relative importance of the entire problem.

Spread of Lesions in Excised Tissue

It had been unofficially reported (Wharton, 1943) that the individual lesions continued to increase in size and undergo degeneration in fillets under the usual conditions of storage. It was further reported that non-infected flesh could become parasitized from contact with contaminated cutting tables, knives, or other objects, and that such fillets would develop lesions during storage. It was necessary, therefore, to investigate these claims under controlled conditions.

Methods of Eliminating Parasites

The problem of whether it was necessary to discard entire fish which showed evidence of parasitism or only those portions which showed lesions had to be considered. Aesthetically, it was desirable to cut out any gross lesions, following the same technic as that used in the rosefish industry. If it were shown, however, that even the clean areas of infected fish were toxic, then the entire fish would have to be discarded. The answer to this rested upon the findings regarding the toxicity of infected specimens.

Toxicity Studies

It had been rumored that parasitized oceanpout were toxic when eaten. This had been spread to a point where one large retailer had refused to make any further purchases of the fish. The experimental evidence upon which these rumors were based was rather flimsy. In one case, three dogs had been fed some of the parasitized fish. Two of them had shown evidence of a mild gastroenteritis following the meal. As there had been no previous controlled feedings and no attempts had been made to free these animals of intestinal parasites prior to the test meal, the results are of doubtful value. In addition to these feeding tests, one fish had

been eaten by a workman who complained of a gastro-intestinal disturbance the day following its ingestion. Again, no controls were available and, in this particular case, it was not known whether or not the fish was parasitized. This presumptive evidence of toxicity, however, made it necessary to conduct feeding tests under controlled conditions.

EXPERIMENTAL METHODS

Pathology

The pathology of infected fish was studied grossly and microscopically by the usual methods. Sections for histopathological examination were stained by Mallory's method.

Detection of Parasitic Lesions

Following the methods developed for parasitism in rosefish, a portable candling device was built1/. This consisted of a wooden box, 18 inches long, 6 inches wide, and 6 inches deep. The box housed four 50-watt bulbs mounted directly over a polished metal reflector which rested on the bottom. The top consisted of a window of frosted glass. A cover was provided to protect the glass in transit. Ventilating holes were bored in the sides to prevent overheating. The entire assembly, with 25 feet of extension cord, weighed eight pounds and could easily be carried and set up in the field.

Fillets were placed upon the glass surface and examined by transmitted light. It was necessary to examine both sides of the strip of flesh in order to detect lesions 1 to 5 millimeters in diameter. Very careful observations were necessary in the case of these small lesions since they were frequently embedded in the tissue and were not readily seen by the casual observer. Larger lesions, over 10 mm in diameter, were easily detected without candling.

Incidence of Infection

The boats were met at the dock and the captains interviewed as to where the catch was made and any other pertinent information. The catch was followed to the processing plant and the number of fish in the haul determined by actual count. As the fillets came from the cutters, they were candled and records made of all fish showing evidence of parasitism. Field studies were arranged to permit determination of the sex of the parasitized fish.

Where it was not possible to cover an entire catch or when too many boats landed fish simultaneously, the captains were interviewed and samples were sent to the College Park Laboratory for examination. Such samples consisted of 100 pounds of round fish. Fillets were cut and candled and the meat used for further experiments.

1/ The authors wish to express their appreciation to Mr. Walter Rust of the laboratory staff for designing and building this piece of equipment.

Spread of Lesions

Two types of experiments were conducted to study the growth of parasitic lesions. Individual lesions were observed over a period of storage for changes in size, appearance, and consistency. Transmission experiments were conducted by placing non-infected fillets in direct contact with infected flesh and by following changes in each fillet during a period of storage. The conditions of storage were comparable to those used in commercial cold storage.

Toxicity Tests

Toxicity tests were conducted by feeding parasitized fish to newly weaned kittens and pigs. The animals were conditioned by elminating intestinal parasites and by allowing the animals to become in good physical condition prior to starting the feeding tests. The amounts of oceanpout fed always exceeded the quantity which might be consumed by a human. This was done to permit any toxic factor to have ample opportunity to act upon the animals. Control animals received equal quantities of non-parasitized fish.

During and following the feeding tests the animals were carefully observed for any evidence of gastro-intestinal disturbance. Body temperatures were taken regularly in order possibly to detect less obvious toxic effects. Stools were examined for excessive mucus, blood, etc. At the end of the feeding tests, the animals were destroyed and careful pathological examinations were made of the individual test animals.

RESULTS

Pathology of Parasitized Fish

The lesions produced by this parasite of the oceanpout are usually located in the anterior third of the musculature, although they may be scattered throughout the entire length of the fish. They are most likely to be located next to the spinal column, but there is no regularity in this respect. They vary in size from less than one millimeter to an area involving almost one-fourth of the muscle tissue on one side of the fish. Either one or both sides of the fish may be infected and in some cases multiple lesions may be seen scattered throughout all of the muscles.

In the early stages the lesions are usually firm and chalky white. Individual "cysts" are arranged parallel with the muscle fibers. Older lesions vary from a cream color to rusty brown and are firm. More advanced lesions are soft and caseous and when very advanced become semi-fluid "pus" pockets. Figure 4 shows moderately advanced, extensive lesions in two specimens of oceanpout.

In none of the fish examined thus far has it been possible to detect the disease by examining the round fish. Only by skinning the specimen and cutting into the flesh can the lesions be seen. The viscera do not appear to be infected.

Histologically, the lesions appear to be heavy-walled "cysts" surrounded by connective tissue. Each "cyst" is filled with numerous sporoplasms. In older

lesions the sporoplasms are larger and multinucleate. At this stage, the "cyst" apparently ruptures and the individual sporoplasms spread out into the surrounding tissues where they, too, rupture, releasing numerous spore-like bodies. The ruptured "cysts" suppurate and apparently break-down completely, leaving very little of the internal structure intact, except for a few occasional sporoplasms.

Although microscopic examination reveals forms which resemble those seen by Robertson (1909), there was no evidence of hyphae as reported by Plehn (1924) or Fish (1934). When stained by Mallory's method the parasites appear as red or reddish-brown units, a staining reaction which is characteristic of the protozoa. Characteristic microscopic fields are shown in figure 5.

Detection of Lesions in Fillets

Fischthal (1944) found that careful candling was necessary to detect parasitism in the fillets which he examined. In this investigation there was little difficulty in detecting lesions 10 mm. and over in diameter, although it was necessary to examine the fillets very closely on both sides in the case of the smaller lesions. Recandling of commercially candled fish revealed that about 60 percent of the parasitized fillets had been overlooked. In one group of 31 fillets packed for consumer use, all by 2 showed evidence of parasitism when re-candled in the laboratory. This was an extreme case, but it indicates the extent to which infected fish may reach the market with the parasites in situ. A very superficial survey of the oceanpout for sale in two retail stores revealed that the consumer occasionally was being sold fish which exhibited large lesions, readily detectable without candling.

Incidence of Parasitism

Field studies were conducted during March, April, and May of 1943. An examination was made of several thousand specimens of oceanpout caught in the waters from Cape May, N. J., to the northerly tip of Cape Cod, Mass. The percentage of fish which were parasitized varied from 4 to 38 depending upon the location of the catch. As the field observations have not been continued, it is not known whether the values for any given area are constant or merely indicate the incidence of parasitism at the time of sampling.

The lowest incidence was observed off the southern shore of New Jersey and increased in the more northerly water. Extensive sampling in the area from the eastern end of Long Island to Nantucket showed that parasitized fish comprised from 4½ to 38 percent of those examined, the greater number of infected fish being taken from the deeper water (fig. 6). The areas of lowest infection were those adjacent to Block Island and in Muskeget Channel.

It is difficult to attach much significance to a single field sampling. During 1944, no field determinations were made but it was reported by several dealers who handled large quantities of oceanpout that the number of infected fish was much lower than in 1943. If this is true, it may be that the parasite displays a cyclic infection pattern but sufficient data are not available, at present, to make a reliable interpretation.

A correlation seemed to exist between the size of the lesion and the degree of infection. In areas where the incidence of parasitism ranged from 27 to 33 percent, the majority of the lesions were 15 mm. or over in diameter, whereas in areas where the incidence was below 27 percent, most of the lesions were under 15 mm. It was also observed that in zones of high incidence, multiple lesions were present in most of the parasitized fish, but single lesions predominated in specimens taken from waters where the lower incidence prevailed.

Apparently the degree of infection was also correlated with the age of the fish (see Clemens, 1920, regarding age). Of one catch of small oceanpout (12 to 16 inches; 5 to 7 years old) taken from Ipswich Bay, only 5 percent were parasitized. None of these exhibited large, localized lesions but all showed multiple, miliary foci scattered throughout the muscle. In specimens ranging from 16 to 18 inches (7 to 10 years old), the infection incidence was 14 percent; for those over this size (10 to 20 years old), it was 25 percent.

The sex of each fish was determined in most cases but there was no apparent difference in the degree of parasitism between sexes. However, individual catches occasionally showed a higher parasitic incidence in one or the other of the sexes.

Spread of Lesions in Fillets

The results obtained in the experiments that were conducted to determine the increases in areas of lesions in fillets clearly show that even with careful candling and proper storage, lesions will continue to develop. The data also indicate that flesh which appeared to be normal upon candling may develop lesions during storage.

The data in table 2 show the results obtained when fillets containing a single lesion each are stored at 8° F. From 48 to 70 percent of the lesions showed a definite increase in size when held in storage from 4 to 14 days. Degeneration, i.e., discoloration and suppuration, occurred in from one-third to one-fourth of the fillets. In a preliminary experiment not recorded in the table, fillets containing miliary lesions were stored for 14 days at the same temperature as those mentioned. Of 40 such fillets, 10 developed "sores" which were readily discernible without candling.

Three storage experiments were conducted with fillets which had been carefully candled and from which all visible lesions had been removed by cutting them out of the meat. These were held at 8° and 40° F. and examined at intervals throughout a period of 7 to 10 days. The data in table 3 show that despite the care taken to remove all of the infected areas which could be located with the candling device, evidences of parasitism appeared after the first day of storage and the number of fillets showing such manifestations increased with the period of storage. By the end of one week nearly all of the fillets exhibited parasitic lesions when examined by candling.

To determine the possibility of contaminating normal flesh by contact with infected material, individual test fillets, which showed no evidence of parasitism, were placed in direct contact with parasitized fillets and each respective pair was

wrapped in parchment paper. An equal number of pairs of apparently non-infected fillets were stored under identical conditions, as controls. The temperature of storage was 8° F. in all cases. In the first experiment, 10 such pairs and an equal number of controls were kept for a period of one week. At the end of this time, there were lesions in 2 of the 10 test fillets and in 4 of the 20 controls. A second experiment using 25 pairs of fillets was conducted (see table 4). These were examined periodically over a period of 7 days. Although lesions did appear in the control series, the degree of parasitism in the test fillets was much higher.

Toxicity Studies

The results of the feeding tests employing cats and pigs indicate that there is very little, if any, danger to their health in eating oceanpout parasitized with Ichthyosporidium. Six young cats, divided into two groups of two and four each, showed no evidence of any ill effects when fed large quantities of raw and cooked (boiled for 30 mins.) diseased fish, respectively. The fish ration was supplemented with reconstituted dried whole milk in all cases, and the animals were maintained on the fish-milk diet for a two-weeks test period. At the end of this time they were destroyed, and a careful post-mortem examination was made of them[2/]. The gross pathology and histology of representative tissues were normal in every respect.

Throughout the feeding tests, body temperatures of the cats were taken regularly, and in no case was an abnormal value obtained. Observation of the animals during and following each test meal failed to reveal any indication of gastro-intestinal distress; vomiting and diarrhea were absent in every case.

In addition to the feeding experiments with cats, six pigs, each weighing about 60 pounds, were subjected to a similar experiment. These were divided into three groups of two animals each. Group one received a single daily feeding of two pounds of uncooked parasitized fillets with a regular grain ration. Group two received the same diet except that the fillets had been boiled for 30 minutes. Group three was fed raw fillets of non-parasitized oceanpout with the grain supplement. The test feedings were given daily for a period of 5 days after which the animals were returned to a normal grain ration for four weeks. Body temperatures were taken periodically throughout the tests and, as with the cats, observations were made for evidences of any gastro-intestinal distress. The animals behaved normally in all respects. Post-mortem examinations revealed only normal tissues in every case.

The findings regarding both the cat and pig feeding tests were summarized as follows: "Post-mortem examinations and histological study of representative tissue did not reveal the presence of any pathological change attributable to the fish feedings" (Coburn, 1944).

2/ The post-mortem examinations were conducted by Dr. Don R. Coburn, Veterinarian in charge of the Patuxent Wildlife Disease Research Laboratory. He also supervised the feeding tests on the pigs and made the post-mortem examination on them.

DISCUSSION

Clemens (1920) examined a large number of oceanpout from the same area for parasites. He reported that only a few worms were present in the flesh. The extensive damage to the host which we have observed plus the above observation indicate that Ichthyosporidium may be a recent parasite of this fish. The infection is too obvious to have been overlooked by Clemens and a well established parasite would be more adaptable in its behavior in the host.

The portal of entry of the parasite is not known but since it is an infection of the deep muscle and no external evidence of parasitism is seen, it is doubtful that it gains entrance through the skin. Caullery and Mésnil (1905 a) described closely related genera in the annelid worms. Since these worms constitute part of the diet of Zoarces, it may be that they serve as the primary source of the infection. If this is true, one might expect the viscera to be parasitized but this does not appear to be the case.

From a strictly practical viewpoint it is almost impossible to eliminate the parasite from fillets, at present. Should the high incidence of infection continue, normal fillets are certain to become contaminated during processing, and storage will permit the organism to develop. It is true, of course, that in none of the experiments was the fish subjected to quick freezing, nor were they held at extremely low temperatures. It may be that a commercial quick-freezing type of treatment might have held the parasite in check or killed it. However, the conditions held during the course of the experiments were probably superior to those employed in plants that do not produce frozen fish, and the results reflect what may be expected in ordinary commercial practice.

SUMMARY

1. An Ichthyosporidian parasite of the oceanpout (Zoarces anguillaris) has been studied in regard to the following: pathology of the parasitized fish, methods of detecting lesions, incidence of infection, spread of lesions in fillets, methods of eliminating lesions from fillets and toxicity of parasitized fish.

2. The incidence of obvious parasitism in oceanpout caught in the North Atlantic waters of the United States in 1943 varied from 4 to 38 percent depending upon the location.

3. Lesions appear to continue to increase in size and number in fillets stored at 8° F. for two or more days. Normal appearing oceanpout flesh developed evidences of parasitism after contact with infected fillets.

4. Feeding tests with cats and pigs revealed no indication of toxicity from the ingestion of parasitized oceanpout.

Table 1. Landings of Oceanpout During 1943

Month	New Bedford		Gloucester, Boston and Portland	
	Pounds	Value	Pounds	Value
January	6,940	$144.00	-	-
February	395,197	15,459.00	12,230	$570.00
March	983,400	27,798.00	33,315	1,287.00
April	1,424,377	49,432.00	43,220	765.00
May	369,215	7,224.00	19,090	303.00
June	-	-		
July	-	-		
August	-	-		
September	-	-		
October	1,045	63.00		
November	615	16.00)		
December	52	2.00)	7,235	232.00

Note:--Total receipts on New York market: 830,333 lbs.

Table 2. Changes in the Size of Individual Parasitic Lesions in Oceanpout Fillets Stored at 8° F.

Experiment No.	Number of fillets with single lesion	Period of storage	Average size of initial lesions	Average size of lesions after storage	Lesions showing increase in size	Lesions showing degeneration 1/ during storage
		Days	Millimeter	Millimeter	Percent	Percent
1	46	4	10 x 16	13 x 19	48	33
2	31	9	16 x 10	-	70	-
3	38	14	12 x 14	14 x 32	58	24

1/ Discoloration and "pus" formation.

Table 3. Development of Parasitic Manifestations in Fillets Initially Freed of lesions.

Number of lesion-free fillets	Temperature of storage °F.	Number of fillets showing lesions after storage					
		0 day	1 day	2 days	3 days	5 days	7 days
25	40	0	5	9	-	12	21
25	8	0	3	7	-	13	24
25	8	0	2	9	11	16	22

Table 4. Transfer of Parasitic Lesions from Infected to Non-infected oceanmout fillets.

	Number fillets employed	Temperature of storage °F.	Number of fillets showing lesions after storage				
			1 day	2 days	3 days	5 days	7 days
Test fillets 1/	25	8	6	14	22	24	24
Control fillets	50	8	1	3	13	-	16

1/ Lesion-free fillet in contact with parasitized fillet.

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Plates I and II

(Copied from Robertson, 1908)

Fig. 1. Shows a medium sized Ichthyosporidium enclosed in a nest of connective tissue, the protoplasm is vacuolated towards the centre. Through an error in drawing, the nuclei have been made too small.

Fig. 2. Ichthyosporidium with homogeneous protoplasm, and large evenly distributed nuclei.

Fig. 3. Parasite issuing from its cyst. The nuclei are arranged towards the centre of the animal. The protoplasm shows vacuoles.

Fig. 4 and 5. Division of the nuclei.

Fig. 6. Group of young individuals.

Fig. 7. Exit of parasite from its cyst.

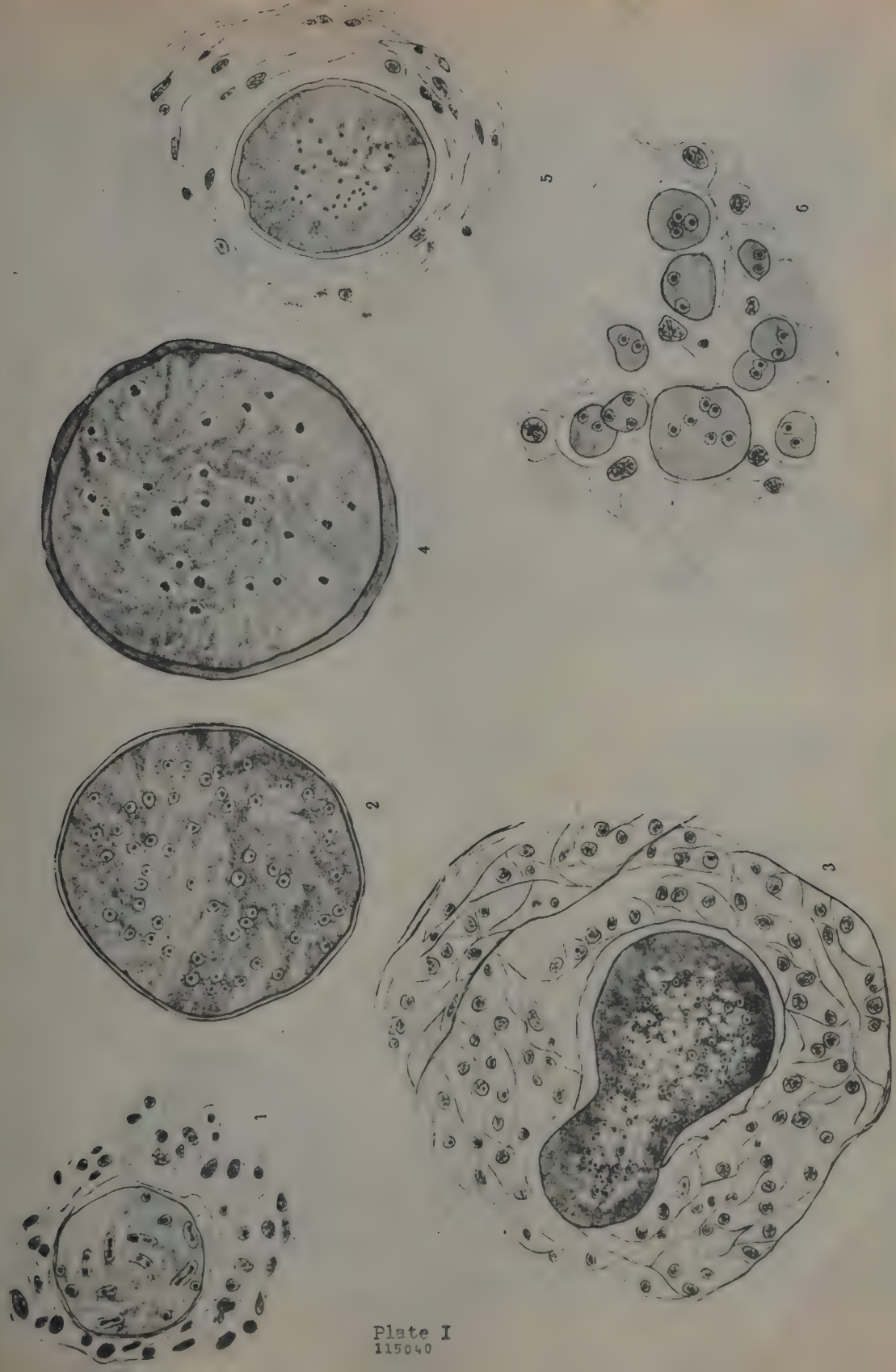
Fig. 8. Abandoned cyst filled with connective tissue.

Fig. 9 and 10 illustrate the breaking up of an individual into reproductive bodies.

Fig. 11. Shows the results of this breaking up process. The young individuals have already begun to grow.

Fig. 12. Section through a nodule with one of the large worm-like individuals. It has been cut in several places, thus giving the appearance of several separate creatures.
At P a stage in the process of plasmotomy may be seen.

Fig. 13. Early stage in the exit of one of these large forms from the cyst.



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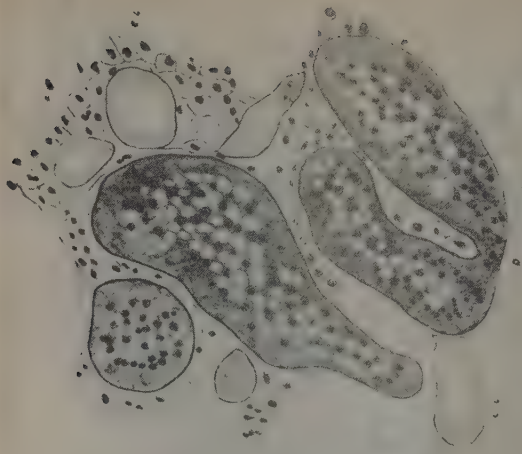

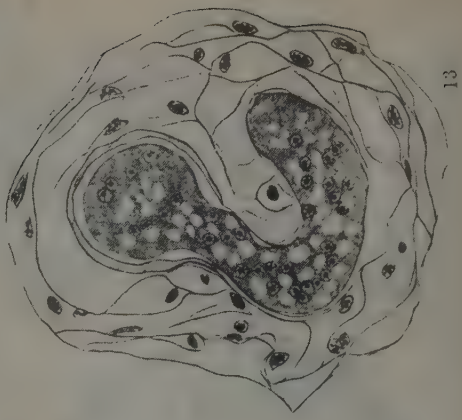

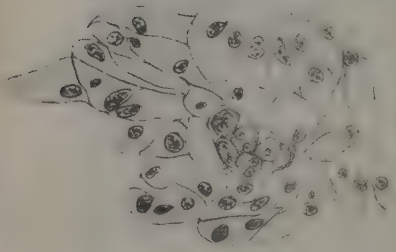

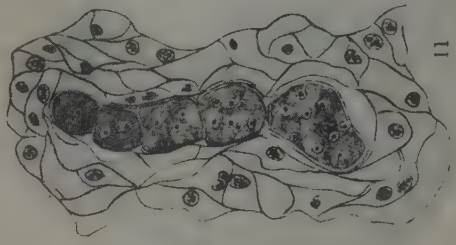

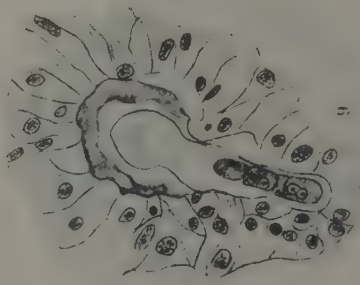

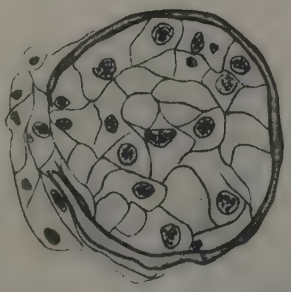

Plate II

Plate III

Life Cycle of *Ichthyosporidium giganteum*
(after Kudo)

Fig. 1 - 5 Schizogony
The amoebulae grow and their nuclei multiply forming plasmodia. The plasmodia divide into smaller bodies while the nuclei continue to divide.

Fig. 6-13 Sporogony
The nuclei become paired (6-7) and the nuclear membranes disappear (8). The plasmodia break up into numberous small bodies, each of which contains one set of paired nuclei (9). This is the sporont (10) which develops into 2 spores by further differentiation (11-13).

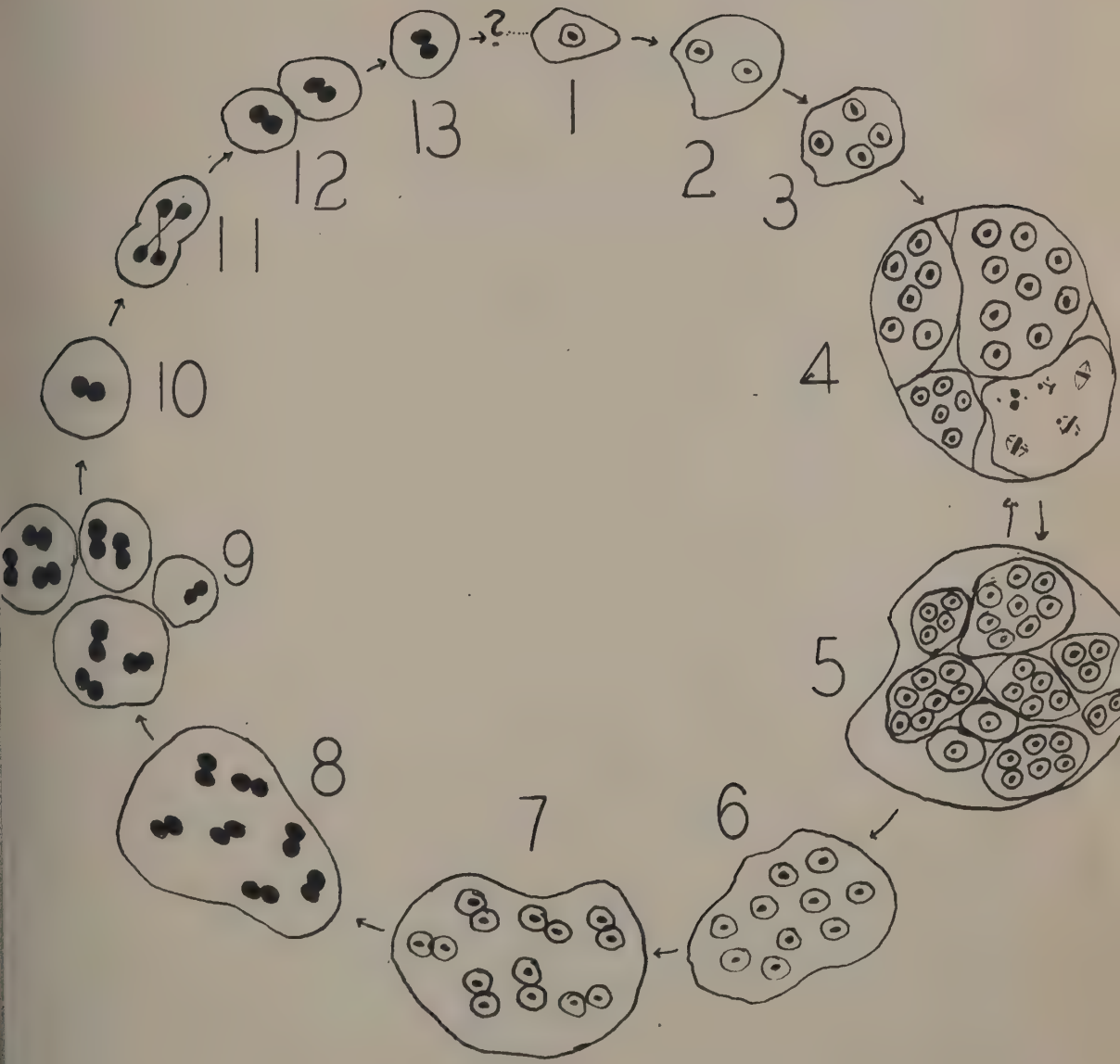

Plate III
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Plate IV

Ichthyosporidian lesions in oceanpout.

The fish have been skinned and the outer muscle layer removed. The lesions appear as white areas in the anterior part of the specimens.

Plate V

Stages in the Development of *Ichthyosporidium* in the Oceanpout

A. Early stage. (200 X)
The parasites (dark, round bodies) are scattered between the muscle fibers. This stage corresponds to #1 in Figure 3.

B. Cyst (200 X)
The plasmodia multiply and are multinucleate. The mass is walled off by connective tissues forming a cyst.

C. "Ripe" cyst (200 X)
The plasmodia have ruptured releasing the sporonts within the cyst wall.

D. Advanced cyst.
The connective tissue wall has ruptured releasing the sporonts which develop into plasmodia, completing the cycle.

E. Resting stage of sporont (500 X)

F. Ruptured and advanced sporonts (500 X)

Plate V
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Plate VI

Map showing the percent incidence of Ichthyosporidian infection in oceanpout caught in the area from Long Island to Nantucket in March, April and May of 1943.

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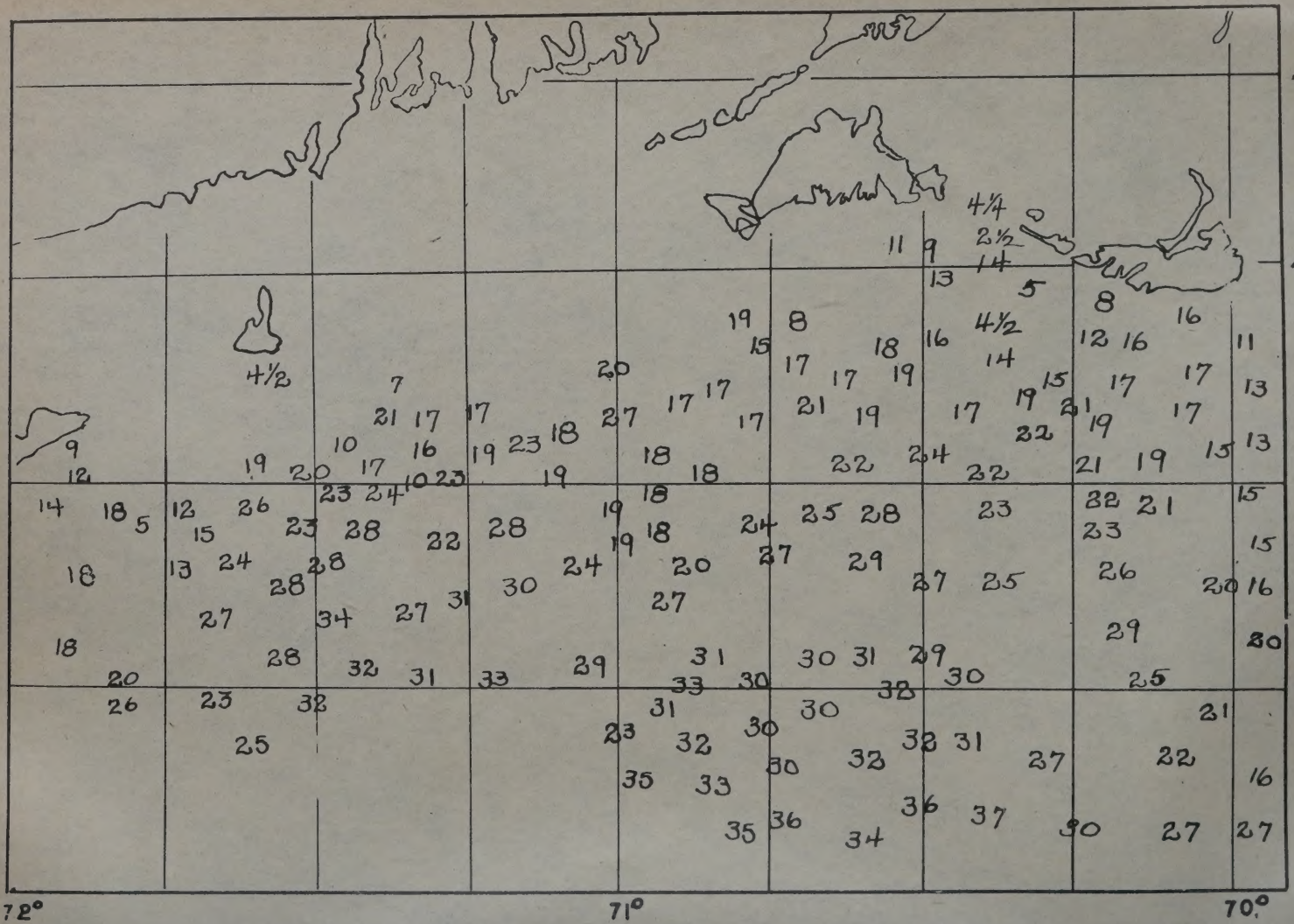

Plate VI
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